Diphtheria in the 1990s: Return of an old adversary

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Readers are invited to use this article as a self-assessment exercise and to update their knowledge.

ILLUSTRATIVE CASE HISTORY

In 1995, a 46-year-old man presented with toxigenic diphtheria of the nasal cavity, pharynx, larynx and trachea. It was not known whether he had ever been immunized but, certainly, no booster immunization had been given during the previous 30 years.

The patient was ill for 5 days before admission to hospital. The first symptom was a sore throat, but no medical advice was sought. After 2 days, his sore throat worsened, his temperature increased and he noticed swelling of the tonsillar region and a nasal discharge; 4 days later, he had marked swelling of the neck. On the night before admission, he was unable to sleep. On admission, he had a serosanguineous nasal discharge and inflammation of the palate, pharynx and tonsils, with a whitish membrane extending to the uvula. The larynx and vocal cords could not be visualized. There was hyperemia of the face and neck with soft tissue edema down to the level of the clavicles as well as tachypnea, tachycardia and arterial hypertension with respiratory distress, indicating respiratory obstruction and respiratory insufficiency. A diagnosis of diphtheria was made. Throat and nasopharyngeal swabs including particles of membrane were taken for culture. These later yielded toxigenic *Corynebacterium diphtheriae* var *gravis*.

Because the clinical picture strongly suggested diphtheria, specific treatment with antitoxin and antibiotics was initiated immediately. The skin test for sensitivity to horse serum was negative. Diphtheria antitoxin (120,000 units) by intravenous infusion over 60 min and intramuscular benzylpenicillin (30 mg/kg/day) was administered. Central venous cannulation was performed for rapid administration of blood products and fluids. In addition, the patient was given corticosteroids, diuretics and dopamine.

Immediately after admission, oxygen was administered, but increasing tissue edema with airway obstruction necessitated emergency airway management. Attempts at intubation by experienced personnel were unsuccessful and tracheostomy was performed, but the tracheostomy was only partially effective because of airway obstruction caused by expansion and sloughing of the tracheal and bronchial epithelium. Bronchoscopy with visual control and evacuation of membrane and other material produced considerable improvement in the airway.

The systemic effects of diphtheria began to appear within a week. Severe myocarditis was diagnosed by physical examination, chest x-ray, electrocardiogram and laboratory data (elevations of serum creatine kinase, aspartate transaminase and lactate dehydrogenase). The main clinical problems were bradycardia and cardiac output disturbances. At the same time, the patient showed manifestations of neuritis with paralysis of the soft palate, the muscles of accommodation, the diaphragm and the limbs. Nutrition was maintained via nasogastric tube during the period of soft-palate paralysis, and mechanical ventilation was introduced at the stage of bulbar disturbance and diaphragm paralysis.

Following termination of the acute infection, management of this patient resolved into adequate support of the vital functions, such as ventilation, nutrition and metabolism, and prevention of nosocomial infection. Six weeks after admission, mechanical ventilation was stopped, although a further 2 months was needed for the neurological signs to disappear. The patient was discharged from hospital 3 months after admission and, 6 months later, physical examination

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Accepted 25 October 1995

revealed no abnormality, in particular, no neurological signs. Nine months later, the patient visited hospital for a final physical examination and vaccination.

MULTIPLE-CHOICE QUESTIONS

Clinical and laboratory aspects of diphtheria

In each of the numbered questions, at least one, and up to four, of the individual entries are correct. (The answers are at the end of this article.)

1. The following *Corynebacterium* species may produce diphtheria toxin

a) *C. diphtheriae* var *mitis* True/False
b) *C. diphtheriae* var *belfanti* True/False
c) *C. ulcerans* True/False
d) *C. pseudotuberculosis* (*C. ovis*) True/False
e) *C. pseudodiphtheriticum* (*C. hoffmanii*) True/False

2. Laboratory diagnosis of diphtheria

a) All potentially toxigenic corynebacteria produce cysteinase. True/False
b) All potentially toxigenic corynebacteria produce pyrazinamidase. True/False
c) Isolates biochemically confirmed as *C. diphtheriae* var *gravis* are always toxigenic. True/False
d) Laboratory-acquired diphtheria has been reported. True/False
e) Genotypic methods for toxigenicity testing [such as polymerase chain reaction (PCR) for the toxin gene] may completely replace phenotypic methods (such as the Elek test). True/False

3. Diphtheria

a) May occur in subjects who have received a full primary course of immunization and booster in childhood. True/False
b) May be caused by *C. ulcerans*. True/False
c) Should be considered in the clinical and laboratory diagnosis of skin ulcers in travellers returning from Asia and Africa. True/False
d) Has an incubation period of not less than 10 days. True/False
e) Soft-palate paralysis, a manifestation of diphtheritic neuritis, usually occurs during the first week of illness. True/False

4. Diphtheria in Russia, the Ukraine and other newly independent states (NIS) of the former USSR

a) These countries reported over 40,000 cases of diphtheria to the World Health Organization (WHO) in 1994. True/False
b) These countries reported approximately 500 deaths due to diphtheria to the WHO in 1994. True/False
c) Between 100,000 and 200,000 diphtheria cases are predicted for 1995. True/False
d) Is almost exclusively a childhood disease. True/False
e) Has not spread beyond the borders of the former USSR. True/False

5. Management of a case of suspected diphtheria

a) Specific treatment should be delayed until laboratory confirmation is available. True/False
b) Diphtheria antitoxin is a hyperimmune human immunoglobulin preparation. True/False
c) Recommended antibiotic treatment regimens include penicillin or erythromycin. True/False
d) Patients recovering from diphtheria should be immunized with diphtheria toxoid before discharge from hospital. True/False
e) Strict isolation of a case is not necessary after 24 h of appropriate antibiotic therapy. True/False

6. Management of close contacts of a case of diphtheria

a) Close contacts include all staff and pupils of a school in which a case has been diagnosed. True/False
b) Close contacts should be kept under daily health surveillance for 7 days from the date of last contact with the patient. True/False
c) In addition to nose and throat swabs from close contacts, any wounds or skin lesions should also be swabbed. True/False
d) Intramuscular benzathine penicillin is the recommended antibiotic of first choice for chemoprophylaxis in close contacts. True/False
e) Booster immunization of close contacts is unnecessary if there is a documented history of diphtheria toxoid immunization in the preceding 5 years. True/False

7. Diphtheria immunity and immunization

a) The vast majority of adults in Western Europe are immune to diphtheria on the basis of serological surveys. True/False

b) Standard pediatric vaccine preparations are suitable for booster immunization in adults. True/False

c) An antitoxin level of < 1 IU/mL in human serum, as determined by a toxin neutralization assay, indicates susceptibility to infection. True/False

d) The WHO has recommended that, by 1995, every country should achieve a 95% coverage for a booster dose of a diphtheria toxoid vaccine in school-age (5 to 14 years) children. True/False

e) High and sustained population coverage with diphtheria toxoid-containing vaccines has led to the virtual disappearance of toxigenic strains in some countries. True/False

COMMENTS

Question 1

Isolates of *C. diphtheriae* var *mitis*, *intermedius* and *gravis*, *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis* may all secrete a potent toxin called diphtheria toxin [1,2] and, thus, may be referred to as potentially toxigenic corynebacteria. Toxigenic and non-toxigenic variants of the three potentially toxigenic species and biotypes are distinguishable by in vitro and in vivo laboratory tests. The nitrate-negative variant of *C. diphtheriae* var *mitis* — *C. diphtheriae* var *belfanti* — is almost invariably non-toxigenic, although there has been one report of a toxin-producing isolate [3]. Diphtheria toxin is a potent polypeptide exotoxin of molecular weight 58 kDa comprising two fragments. Fragment B binds to specific receptors on susceptible cells whereas, following proteolytic cleavage, fragment A (the 21 kDa N-terminal fragment) passes into the cytoplasm. Fragment A is an enzyme which catalyzes inactivation by adenosine diphosphate (ADP) ribosylation of the eukaryotic transfer ribonucleic acid (tRNA) translocase (elongation factor 2). At a minimal lethal dose of < 0.1 μg/kg body weight, diphtheria toxin is one of the most powerful bacterial toxins and is responsible for the clinical manifestations of the disease. Toxigenicity is correlated with infection of *C. diphtheriae* by a temperate phage, and lysogenization of a non-toxigenic strain with phage carrying the toxin gene will convert the organism into a toxigenic strain. The toxin is therefore, by definition, a phage-encoded protein [4].

Corynebacterium ulcerans is usually a commensal, and *C. pseudotuberculosis* is usually a pathogen, of farm animals, in particular, cattle and horses. Both species may produce diphtheria toxin [2] in addition to a dermonecrotic toxin. However, *C. ulcerans* has been associated with diphtheria-like illness in humans [5,6] whereas, although responsible for rare cases of suppurative granulomatous lymphadenitis [7], *C. pseudotuberculosis* has not. Exposure to infected animals or animal products, in particular, unpasteurized milk, appears to be necessary for infection, and person-to-person transmission of these species has not been described. In contrast, *C. pseudodiphtheriticum* does not produce any toxins and is considered part of the normal human pharyngeal flora [8].

Question 2

The principal role of the microbiology laboratory in the diagnosis of diphtheria is to provide simple, rapid and reliable methods to assist the clinicians in confirming a clinical diagnosis. Microbiological diagnosis must be regarded as only complementary to, and not a substitute for, clinical diagnosis. Throat, nose and skin ulcer/wound swabs should be plated onto a non-selective blood agar and a selective medium containing potassium tellurite and lysed horse blood such as Hoyles medium. Suspicious (black on tellurite-containing media) colonies should be Gram-stained and tested for catalase and urease production. Whereas *C. diphtheriae* biotypes are catalase-positive, urease-negative, gram-positive rods, *C. ulcerans* and *C. pseudotuberculosis* are urease-positive [9].

Two important tests for differentiating the potentially toxigenic corynebacteria from the non-toxigenic species involve detection of the enzymes pyrazinamidase and cysteinase. Cysteinase production is readily detected on Tinsdale medium: *C. diphtheriae*, *C. ulcerans* and *C. pseudotuberculosis* yield black colonies with a surrounding brown halo of diffused pigment whereas other corynebacteria may produce black colonies, but without a brown halo. It is advisable that each plate of Tinsdale medium be controlled by a clearly demarcated, small section of the plate that is heavily inoculated with a known cysteinase-positive strain. Pyrazinamidase production is detected by incubating a dense suspension of the organism in a sterile solution of pyrazinamide for a minimum of 4 h at 37°C and then adding ferrous ammonium sulfate solution. A negative result, absence of pyrazinamidase production, is indicated by a colorless to pale-yellow reaction and a positive result by the development of a deep orange-to-red colour. The potentially toxigenic species are all negative for pyrazinamidase production and all other corynebacteria are positive. As a negative

result is potentially of great significance, it is always advisable to set up a known pyrazinamidase-positive control strain, such as *Corynebacterium xerosis* (NCTC 12078), at the same time [9,10].

The definitive identification of *C. diphtheriae* to species and biotype level, and other corynebacteria to species level, relies on biochemical tests, fermentation of sugars, hydrolysis of urea and nitrate reduction in addition to the detection of toxigenicity. All biotypes of *C. diphtheriae* except *belfanti* are potentially toxigenic, but there is no absolute association between the biotype *gravis* and the ability to produce diphtheria toxin; consequently, it is necessary to subject all isolates to toxigenicity testing [9,10]. The Elek test is the only available method in the majority of laboratories. Given the immense public-health significance attached to the isolation of toxigenic *C. diphtheriae*, any delay between isolation of a suspect organism and the results of toxigenicity tests can provoke great anxiety among laboratory staff, clinicians and public-health officials. The development of a rapid genotype test, using PCR for detection of the toxin gene, provides a rapid and useful assay of the potential for toxigenicity [11]. However, a small number of isolates have been reported which, although possessing the toxin gene, are unable to express the gene product [11]. Thus, a definitive demonstration of toxigenicity still requires some form of phenotype test.

At least one case of laboratory-acquired diphtheria has been reported [12]. It is therefore recommended that laboratory workers who will or may handle toxigenic isolates should be proven to be immune by serological testing and receive booster immunization if necessary. In addition, it is reasonable to handle all work involving broth cultures or liquid suspensions of actual or potentially toxigenic strains in a class 1 biological safety cabinet. It should be remembered that the Elek test requires control with both strongly and weakly toxigenic *C. diphtheriae* control strains and, thus, even in the absence of toxigenic clinical isolates, laboratory workers are at risk.

Full details of these methods are given in the Manual for the Laboratory Diagnosis of Diphtheria, published recently by the WHO Regional Office for Europe [9].

Question 3

Immunity to diphtheria wanes over time and, consequently, the disease may occur in adults with a full and documented history of childhood immunization. An illness consistent with a clinical diagnosis of diphtheria in a fully immunized subject should be regarded as such until proven otherwise.

Corynebacteriym ulcerans may produce diphtheria toxin and, thus, may produce a clinical syndrome indistinguishable from disease due to toxigenic *C. diphtheriae*. Symptoms and signs of toxigenic *C. ulcerans* infection include exudative pharyngitis with toxic cardiac and neurological manifestations [5,6]. In suspect cases, diphtheria antitoxin should be administered.

Cutaneous diphtheria should be considered in travellers returning to Europe from countries in which diphtheria remains endemic, particularly the tropics. The usual presentation is of chronic non-healing ulcers with a dirty-grey membrane. *Staphylococcus aureus* and *Streptococcus pyogenes* are also often isolated from these lesions. Cutaneous diphtheria does not usually lead to signs of intoxication, although such infections do induce high levels of circulating antitoxin and may act as natural immunizing events. Skin lesions may serve as a reservoir of toxigenic *C. diphtheriae* and may also contaminate the environment. Cutaneous diphtheria will induce throat infections in contacts at least as efficiently as pharyngeal infection [13].

The usual incubation period for respiratory tract diphtheria is 2 to 5 days. Contacts of a case should be kept under health surveillance for 7 days from the time of last contact with the patient [14]. Soft-palate paralysis, the most common neurological manifestation of diphtheria, usually develops during the third week of illness and is characterized by a nasal quality to the voice and nasal regurgitation. In addition, other neurological manifestations include cranial and peripheral nerve palsies that are predominantly bilateral with motor, rather than sensory, involvement, and diaphragmatic paralysis. Provided that the patient survives, the acute effects these neurological manifestations usually resolve completely. In most cases, the cardiac manifestations of diphtheria intoxication appear during the second week of illness. The more extensive the local lesion and the more delayed the institution of antitoxin therapy, the more frequently will myocarditis occur [14].

Question 4

Although diphtheria was controlled for approximately 30 years after the introduction of childhood vaccination with diphtheria toxoid in the late 1950s, epidemic diphtheria has reemerged in the NIS of the former USSR. The epidemic began in 1990 in the Russian Federation and spread to the Ukraine in 1991 and, during 1993 to 1994, to all 13 of the remaining NIS. Overall, reported cases of diphtheria in the NIS increased from 839 in 1989 to 47,802 in 1994. In 1994, a total of 1746 persons died; the case fatality rates ranged from 2.8 to 23% with 80% of these cases occurring within the Russian Federation [15].

Epidemiological analysis indicates that 150,000 to 200,000 cases with 7500 to 10,000 deaths will probably occur in 1995 if the emergency actions proposed to control the epidemic are unsuccessful [16].

Although the reasons for the epidemic are not fully understood, an important factor is the presence of large numbers of susceptible children and adults in the population which has enabled the spread of toxigenic *C. diphtheriae* from foci of endemicity within the former USSR or from Russian military personnel returning from such areas in other countries. Spread of the organism may also be facilitated by crowding and population migration. The increased number of susceptible children in the NIS is probably the result of a combination of low vaccination coverage in many areas and inadequate primary vaccination courses [15]. In Russia in 1993, the rates of disease per 100,000 population did not differ between adults and children (age 0 to 14 years, 12/100,000 population; age > 14 years, 9/100,000 population; all ages, 10/100,000 population) [14].

During the past 3 years, Finland, Germany, Norway and Poland have registered cases imported from countries of the former USSR [14]. Two citizens of the United States contracted the disease after visiting or working in the Russian Federation [17].

Question 5

Specific treatment with antitoxin and antibiotics should be commenced immediately if diphtheria is suspected on clinical grounds. In most countries of Western Europe, where diphtheria is extremely rare, suspect cases or their close contacts are very likely to have a relevant travel history and this should always be sought in such cases. The drugs of choice are penicillin and erythromycin by injection until the patient is able to swallow comfortably. Antibiotic treatment is necessary to eliminate the organism and prevent spread; it is not a substitute for antitoxin treatment. Under no circumstances should specific (antitoxin) treatment be delayed pending laboratory confirmation of toxigenicity if the clinical examination and patient history are suggestive of diphtheria.

Diphtheria antitoxin is a hyperimmune serum produced in horses. Before antitoxin is administered, the patient should be tested for sensitivity to horse serum and, if necessary, desensitized. The dose of antitoxin depends on the site and extent of the diphtheritic membrane, degree of toxicity and duration of illness, and is typically 20,000 to 40,000 units by intramuscular or intravenous injection for pharyngeal or laryngeal diphtheria. However, there are manufacturer and national health authority variations in dosage recommendations, and prescribers should seek expert advice. Epinephrine should be readily available whenever antitoxin is administered in case of acute anaphylaxis. Antitoxin only neutralizes circulating toxin that is not yet bound to tissues and, thus, prompt administration is critical. Delayed administration increases the risk of late effects such as myocarditis and neuritis.

All cases (suspected or confirmed) should be reported to the local health authority without delay, and advice from the national communicable disease surveillance unit and the reference laboratory sought immediately. The patient should be nursed in strict isolation until bacteriological clearance has been confirmed by negative cultures of nasopharyngeal and throat swabs obtained at least 24 h after completing treatment. Clinical diphtheria does not necessarily confer natural immunity and, therefore, patients with diphtheria should be vaccinated before discharge from hospital. Previously unvaccinated individuals should immediately receive a dose of diphtheria toxoid-containing vaccine (preferably Td) and, later, complete a full primary course of no fewer than three doses. Partially vaccinated subjects should complete the primary course according to the national recommendations; fully vaccinated persons should receive a booster dose. More detailed descriptions of the procedures involved are given in the WHO Manual for the Management and Control of Diphtheria [14] and by Farizo and colleagues [18].

Question 6

Anyone who has been in close contact with a case of diphtheria caused by toxigenic *C. diphtheriae* in the previous 7 days should be considered at risk. Contacts of cases due to non-toxigenic *C. diphtheriae* are not at risk. Close contacts include: school-classroom contacts; household contacts; friends, relatives and caretakers who regularly visit the home; kissing/sexual contacts; those who share the same room at work; and health workers exposed to oropharyngeal secretions from the patient [14].

Close contacts should be clinically assessed for symptoms and signs of diphtheria, including cutaneous diphtheria, and kept under surveillance for 7 days from the time of the last contact with the patient. In addition, a travel history should be obtained from contacts as they may be the source of the patient's infection. The management of close contacts includes clinical surveillance, bacteriological investigation (nasal, pharyngeal and wound or other skin lesion swabs), antibiotic therapy and immunization. Carriage rates of toxigenic *C. diphtheriae* among household contacts may be as high as 25%. If a positive culture is obtained from a close contact, the carrier's close contacts should be identified and the preventative measures described for

close contacts of a patient initiated. Recommended chemoprophylaxis regimens include intramuscular benzathine penicillin or a 7- to 10-day course of oral erythromycin. However, although the latter is easier to administer, it is not routinely recommended because of the risk of poor compliance. Repeat culture should be undertaken 2 weeks after completion of the antibiotic course to ensure eradication of the organism.

Close contacts who have received fewer than three doses of diphtheria toxoid in the past or whose immunization status is unknown should be given an immediate booster dose of diphtheria toxoid-containing vaccine, followed by completion of the full immunization series according to the nationally recommended schedule. Contacts who have had three doses of vaccines in the past should also receive an immediate booster dose. If the last dose was given during the previous 12 months, a booster dose is unnecessary [14].

Question 7

Serological surveys have revealed that there is a significant 'gap' in adult immunity to diphtheria toxin. A recent study from the UK of 1000 blood donors, aged 20 to 59 years, showed that, overall, 37.6% were susceptible (antitoxin levels < 0.01 IU/mL) to diphtheria, 31.5% had basic protection (antitoxin levels 0.01 to 0.09 IU/mL) and 30.9% were fully protected (antitoxin levels > 0.1 IU/mL). In this study, there was a significant trend of decreasing immunity with increasing age and, among those aged 50 to 59 years, 53% were susceptible [19]. Similar results have been obtained in serological surveys from other Western European countries and the United States.

Pediatric diphtheria toxoid-containing vaccines are not suitable for booster immunization of adults mainly because such vaccines contain large antigen doses, which are necessary for priming the immune response. Exact details of vaccine composition may vary among manufacturers and depend on national recommendations. However, typical pediatric preparations contain 30 IU of diphtheria toxoid whereas adult booster preparations usually contain 4 IU of diphtheria toxoid. Use of the larger (pediatric) antigen dose for booster immunization in adults and adolescents is more likely to induce adverse reactions and therefore should be avoided.

For epidemiological purposes, the minimum protective level is considered to be 0.01 IU/mL of diphtheria antitoxin in a serum sample. The higher level of 0.1 IU/mL is desirable for individual protection but, in the majority of subjects, such levels are not maintained over a long period of time. An antitoxin level greater than 1 IU/mL is regarded as indicating long-term protection [9]. Periodic serological surveys are essential with particular emphasis on adults over the age of 30 years whose immunity has not been boosted by natural infection.

The targets proposed by the WHO are that each region should achieve 95% coverage with both a primary immunization series in children by 2 years of age and a booster dose of diphtheria toxoid-containing vaccine in school-age children [14]. As part of the WHO strategy for prevention and control of diphtheria in Europe, it has been proposed that countries affected by a resurgence of diphtheria should institute mass immunization with diphtheria toxoid-containing vaccines (preferably Td) for certain 'high-risk' groups, including adults over 25 years of age who are healthcare workers, members of the armed forces, employees of transportation services with frequent public contact, teachers, kindergarten and creche staff, alcoholics and among the homeless [20].

In many countries of Western Europe with effective immunization programs, circulation of toxigenic strains of *C. diphtheriae* has virtually ceased for reasons that are not entirely clear and much debated. In the absence of circulating toxigenic *C. diphtheriae*, 'natural immunization' of populations, including boosting the immune response by exposure to such strains, can no longer take place. Thus, it may be necessary to consider a wider and more systematic application of diphtheria booster immunization among adults to ensure adequate future 'herd' immunity and to avoid the potential reemergence of epidemic diphtheria in the 1990s that has already been realized in countries of the former USSR.

Answers to the multiple-choice questions

Q1: a. True; b. True ; c. True; d. True; e. False
Q2: a. True; b. False; c. False; d. True; e. False
Q3: a. True; b. True; c. True; d. False; e. False
Q4: a. True; b. False; c. True; d. False; e. False
Q5: a. False; b. False; c. True; d. True; e. False
Q6: a. False; b. True; c. True; d. True; e. False
Q7: a. False; b. False; c. False; d. True; e. True

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